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MICROFLORA MODIFICATION IN TEMPERATURE ABUSED SHELLSTOCK OYSTERS

F I N A L R E P O R T

January 1, 1985 through March 31, 1987

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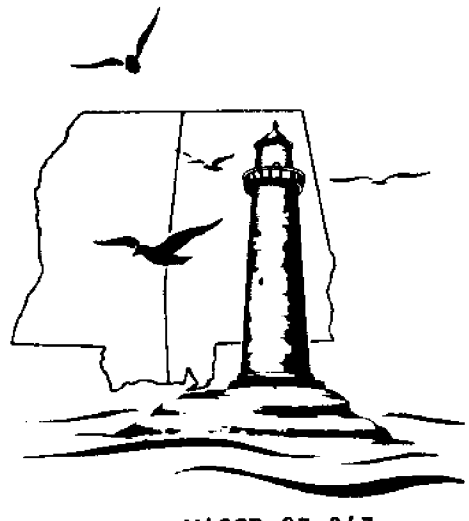
February 1988



MASGP-87-047

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Grant No.: NA85AA-D-SG005C

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Title: Microflora Modification in Temperature Abused Shellstock Oysters

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Date: February 24, 1988

Publication Number: MASGP-87-047

Price: $ 5.00

Publication Abstract

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Project No.: R/MT-10

Program Year(s): 1985, 1986 and 1987.

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INTRODUCTION

The levels of specific types of bacteria in food items have been used to estimate the degree of spoilage or health hazards associated with the food item. In raw oysters, the aerobic plate count (APC) and fecal coliforms have been the bacterial indicators of choice. The fecal coliform group is used as an index of sanitary quality and the APC to indicate improper handling or inadequate refrigeration.

The 1965 revision of the National Shellfish Sanitation Program (NSSP), Manual of Operations, Part I [26] stated the following guidelines for fresh and frozen oysters at the wholesale market level, provided they could be identified as having been produced under the general sanitary controls of the National Shellfish Sanitation Program:

> Satisfactory. Fecal coliform density of not more than 230 MPN per 100 gram and 35°C plate count of not more than 500,000 per gram will be acceptable without question.
>
> Conditional. Fecal coliform density of more than 230 MPN per 100 grams and/or 35°C plate count of more than 500,000 per gram will constitute a conditional sample and may be subject to rejection by the state shellfish regulatory authority.

The above guidelines have been interpreted into legal codes in some states. During the early 1980's, when demands for oysters became high on the East Coast, large quantities of shellstock oysters were shipped there from Louisiana during the summer. Some of these shipments were sampled upon arrival in the receiving states, found to exceed the fecal coliform portion of the guideline, and resulted in rejection or destruction of the

oysters.

In response, the Interstate Shellfish Sanitation Conference addressed this problem at its 1983 and subsequent annual meetings and worked out a protocol to deal with shipments of shellstock oysters which failed to meet the bacteriological guidelines. This protocol was accepted and made part of the 1987 Revision of the NSSP, Manual of Operations, Part II [5]. Further, this manual reaffirmed the bacteriological guidelines for market shellfish as previously stated, but cautions that the presence of fecal coliforms in excess of 230 MPN/100 gm in oyster meats after harvesting may not be indicative of fecal contamination of Gulf Coast oysters harvested during warm periods.

The study reported here was initially planned in 1982 to address the fecal coliform problem as stated, but funding for the project did not become available until January 1985. Therefore, several changes in the way public health officials may view or react to excessive levels of indicator bacteria in shellstock oysters were made during the period of this research. However, the authors feel that this research, which has documented microflora changes in shellstock oysters during movement in commercial channels and under conditions of temperature abuse, may be useful in future interpretations of bacteriological data.

In recent years a number of Vibrio related illnesses and deaths have been documented in which shellfish were the vector for the vibrios. Relatively little is known about how shellfish handling practices and shellfish storage temperatures effect the

levels of these bacteria in oysters.

In this project we have studied the microflora of oysters harvested from approved shellfishing areas and shipped through commercial channels as well as oysters subjected to temperature abuse in an attempt to answer the following questions:

(1) What storage conditions permit fecal coliform bacteria to multiply in shellstock oysters?

(2) What types of fecal coliform bacteria multiply in shellstock oysters under various storage conditions?

(3) Do conditions which permit fecal coliform bacteria to multiply in shellstock oysters also permit vibrios and other potentially pathogenic bacteria to multiply?

(4) Is there a correlation between fecal coliform or *E. coli* multiplication and vibrio or pathogen multiplication?

REVIEW OF LITERATURE

Fecal coliforms have been used extensively as indicators of fecal contamination in many food products. Any indicator bacteria has its limit of usefulness and one of these is when it multiplies under conditions unrepresentative of its natural habitat. That is to say, if the fecal coliforms multiply within shellstock oysters after harvest, their usefulness as an index of fecal contamination in the oysters at the time of harvest is lost.

Presnell and Kelly [36] published an extensive study of aerobic plate count (APC) and indicator bacteria levels in Gulf coast oysters. They found that APC and coliform counts increased in shell oysters between the time they were harvested and when they were shucked. These increases occurred in oysters stored at room temperature (17-28°C) and under refrigeration. Escherichia coli counts were measured on refrigerated oysters only and were not found to increase during 15 days of storage. These workers concluded that E. coli would probably be superior to coliforms as an index of sanitary quality in market oysters. At the 1964 National Shellfish Sanitation Conference, the fecal coliform group was selected as the bacteriological indicator of sanitary significance in oyster meats [26]. Following this, two studies were conducted which focused on the change in fecal coliform counts in shellstock between harvest and processing. Coliform, fecal coliform, E. coli and APC were followed in Alabama oysters from harvest through overnight storage on unrefrigerated shucking

benches. No change in the fecal coliform counts were observed although the coliform counts and APC increased greatly. However, it was reported that the average air temperature on the oyster boat was 67.3°F, indicating that this study was not done during the summer [35]. A subsequent study [3], conducted in Louisiana in August when temperatures exceeded 80°F, presented a different picture. Four of seven lots of shell oysters from one area showed significant fecal coliform increases within 12 hours of harvest and reached counts of >230/100g by the time the oysters reached the processing plant. The remaining three lots showed a large increase in fecal coliform counts when sampled 24 hours after harvest. However, two lots of oysters taken from a second area and held above 80°F for 50 hours failed to increase in fecal coliforms. These results lead the author to speculate that some factor or factors other than time and temperature influenced the patterns of fecal coliform change.

The next major study of fecal coliforms in shellstock oysters was undertaken by the Food and Drug Administration during July, 1983 in Louisiana [4]. Again it was demonstrated that fecal coliform bacteria could multiply in oysters and that handling and transport practices commonly used in the industry permitted the fecal coliform count and APC to increase. Studies of E. coli multiplication were inconclusive because, multiplication occurred in oysters from one harvest area, but not another.

In the report on the Louisiana study [4], FDA concluded "that the results of shellstock sampling cannot be depended upon

to confirm that oysters necessarily came from harvest areas subject to pollution." Therefore, there appears to be little value in applying a fecal coliform standard to shellstock oysters if the purpose of that standard is to indicate a level of fecal contamination in the oysters.

It is not uncommon to find bacteria of human health concern in oysters taken from approved areas. These bacteria include both allochthonous forms (Salmonella [42], Staphylococcus [42], "atypical" mycobacteria [25], Plesiomonas shigelloides [39] and Yersinia enterocolitica [34]) and autochthonous forms (Vibrio parahaemolyticus [21, 23, 42, 46, 47], V. cholerae [22,23], V. vulnificus [23,45] and Aeromonas hydrophilia [23]). The levels of these bacteria found in oysters are usually below the infectious dose for man. However, if the carrier oysters are held under conditions which permit these bacteria to proliferate, a real human health concern may exist.

Consumption of raw oysters containing pathogenic strains of these bacteria has resulted in a number of diseases in the United States. Raw oysters have been implicated in at least one case of gastroenteritis caused by V. parahaemolyticus [38, 44]. The presence of V. cholerae in oysters has been associated with a number of large outbreaks of cholera as well as less severe cases of gastroenteritis [9, 10, 11, 13, 14, 52]. Ingestion of oysters containing V. vulnificus has resulted in primary septicemias in physiologically and immunologically compromised individuals (liver disease, hematopoietic disorders, chronic renal

insufficiency, use of immunosuppressive agents and heavy alcohol consumption) [6, 7, 27, 38].

Several investigators have considered the multiplication of pathogens in shellstock oysters with the following findings. In general, shellstock oysters held above 20°C show an increase in V. parahaemolyticus during the first few days of storage followed by a decline [28, 43, 48]. At temperatures of <10°C, V. parahaemolyticus may remain viable for greater than 14 days. V. cholerae and Lactose positive vibrios both showed increases in stored oysters at temperatures of 8 and 20°C for 7 days followed by a decline as storage continued [23].

The study by Hood et al.[23] considered the fate of a large number of microbial types in shellstock oysters during storage but failed to address the problem from a real time approach. Oyster sampling times were restricted to 0, 7, 14 and 21 days. Data [4, 24, 36, 48] indicates that significant changes occur in the microflora of oysters within a few hours after harvest and by the 7th day, some types which had increased are declining. Also, oyster shellstock is rarely held more than 5 days between harvest and processing.

In summary, the literature suggests that fecal coliforms can multiply in shellstock oysters. If true, this would negate the use of fecal coliform levels in oysters as an index of water quality in the shellfish harvest area. This study was undertaken not only to verify that observation, but also to develop information that may relate the changes in fecal coliform counts

to the concentration of bacteria of human health concern that may be present in the oysters. This information is essential in developing strategies for protecting the health of raw oyster consumers.

MATERIALS AND METHODS

Oyster Harvesting, Sampling and Transport:

Oysters were harvested by oystermen as part of their normal commercial operation in approved shellfish harvesting areas in Louisiana. Gulf Coast Research Laboratory personnel were on board the boat during the harvest to handle the oysters as described below. Typically the oysters were culled and placed in the containers for transport within 30 minutes after harvest. Oysters for both the commercial handling study and the storage study were taken from a single lot of approximately 250 oysters.

Commercial handling studies - Approximately 50 oysters were placed in each of two burlap sacks taken from the supply on the boat and tagged so that they could be easily identified. A temperature recorder was enclosed in each sack. The sacks were placed on different sides of the boat and interspersed with sacks of the commercial harvest. It is typical in the industry for sacks of oysters to remain unrefrigerated on the deck of the boat between harvest and unloading at the dock. After the boats arrived at the dock and just prior to loading the sacks into a truck for transport to a shucking plant in Mississippi, 12 oysters were removed from each sack and placed into an insulated chest and cooled with ice. These samples were maintained at $<10^{o}C$ and were analyzed within 18 hours of collection. The sacks were then retied and placed in the truck so that one sack was in the center of the load and the other on the top of the load. All trucks were equipped with cooling units. On the following

morning, the tagged sacks were recovered from the truck at the oyster shucking plant and transported to the laboratory. There, they were placed under refrigeration until analyzed, usually within 6 hours. On three occasions transport trucks were not available and the oysters were returned to the laboratory in an unrefrigerated truck and placed under refrigeration until analyzed.

At the time of harvest, the air temperature, surface water temperature and the internal temperature of a freshly harvested oyster was measured. When the oysters were removed from the sacks at the dock, the internal temperature of one oyster from each sack was measured with a thermometer. Two surface water samples were taken at the harvest site for bacteriological analysis. These samples were placed on ice and analyzed within 24 hours of collection. The salinity of the water from the harvest area was measured with a refractometer.

Storage studies - Fifty oysters were placed into each of three wire baskets and each basket was in turn placed into an insulated chest. The wire baskets supported the oysters off the bottom of the chest thus preventing them from coming in contact with any liquid that may accumulate. The temperature of each chest was adjusted to <10°C, 22°C or 30°C by adding plastic bottles containing ice or warm water. A battery-operated fan was placed in the chest to circulate the air and facilitate temperature equilibrium. A thermometer and a temperature recorder was added to each chest to verify the temperature. The

temperature in the chest was checked frequently during transport to the laboratory and more ice or warm water was added as needed to keep the temperature constant. Upon arrival at the laboratory, the baskets were transferred to incubators preset to 10°C, 22°C and 30°C. Bacteriological analyses were run approximately 24 hours after harvest and after 3 and 5 days of storage at controlled temperatures.

The selection of the three storage temperatures and sampling times were based on the range of temperatures from the storage conditions (10°C or 50°F) recommended by the NSSP [26], which could be considered the ideal storage temperature, to storage at 30°C or 86°F which could be considered the worst case abuse storage temperature under which the oysters could survive. An intermediate temperature of 22°C or 72°F was set. The time periods of 1, 3, and 5 days were set to approximate lengths of time oysters may be held between harvest and processing. Most oysters are processed within 3 days of harvest but on occasions when oysters are shipped over great distances, they may be held for 5 days. In our experience, most of the oysters held at 30°C died in 5 days so we discarded the five day sampling period at that temperature.

Growth of bacteria in shell liquid - Oysters were removed from Davis Bay near GCRL and held at 25°C for 4 hours. Shell liquid was obtained from oysters by prying the oysters open at the hinge taking care not to rupture the adductor muscle. The

liquid was filtered through sterile glass wool to remove particulate matter and placed in sterile tubes for incubation.

Bacteriological Analysis:

All bacteriological media and media components were Difco brand.

Sample preparation - Shellfish samples for bacteriological analysis consisted of 10-12 oysters. After the shells were scrubbed vigorously with a stiff brush under running tap water and allowed to drain on clean towels, oysters were shucked into a sterile beaker until 200 g of meat were collected. Homogenization was with phosphate buffered dilution water as described in Recommended Procedures for the Examination of Sea Water and Shellfish [2]. All test media were inoculated from a single series of dilutions prepared with phosphate buffered dilution water.

Aerobic plate counts - Aerobic plate counts were done using recommended procedures for a standard plate count of shellfish [2].

Fecal coliform analysis - Fecal coliform and E. coli levels in oyster samples were obtained by a 3-tube Most Probable Number (MPN) technique [2] using lauryl sulfate tryptose broth followed by confirmation with EC broth. A 5-tube MPN was used with water samples. Positive EC broth tubes were streaked onto eosin methylene blue agar, and representative fecal coliform colonies were streaked for purity on plate count agar (PCA) and held for further analysis. Differentiation of fecal coliform isolates was

done by the IMVIC procedure [18], and the Enterobacter and Klebsiella were separated on the basis of motility and ornithine decarboxylase medium [33]. The identification of selected isolates was confirmed with the API 20E system (Analytab products).

Salmonella analysis - Salmonella was measured by a semi-quantitative method, involving the culture of separate 100 g, 10 g, and 1 g amounts of oyster meat homogenate. Isolation procedures [17] included non-selective enrichment in lactose broth followed by selective enrichment in selenite cystine broth and tetrathionate broth. Enrichment broth tubes were streaked onto each of the following selective plating media: bismuth sulfite, Hektoen enteric and xylose lysine desoxycolate.

Typical Salmonella-like colonies were picked from the selective plates and maintained on nutrient agar slants for further testing. Isolates were screened for oxidase, indole, triple sugar iron agar, lysine iron agar, and urease reactions, and when necessary were confirmed using the API 20E system.

Aeromonas analysis - Levels of Aeromonas hydrophila were determined by adding oyster homogenate to alkaline peptone water in a series of dilutions. These enrichment tubes were incubated at 35°C for 18-24 hrs. Tubes with growth were streaked onto Rimler Shotts agar [40] and incubated at 35°C for 18-24 hours. Typical colonies were picked and isolated on T_1N_1 agar [17]. Isolates were then inoculated onto AH-medium [29] and incubated 18-24 hours at 35°C. Cultures giving typical reactions on AH-

medium were tested for a salt requirement as described below. Identifications were confirmed using the API 20E system. Results were reported as the highest dilution where Aeromonas hydrophila was found.

Vibrio analysis - Alkaline peptone enrichment tubes described above were also used to determine the levels of Vibrio cholerae, Vibrio mimicus, Vibrio parahaemolyticus, and Vibrio vulnificus. Each tube was streaked onto a plate of TCBS medium and incubated for 24 hours at 35°C. Typical Vibrio-like colonies were picked from each plate and isolated on T_1N_1 agar. All isolates were maintained on T_1N_1 agar and were screened by the following characterization tests: salt requirements; cytochrome oxidase; presence of B-D-galactosidase; and fermentation of sucrose, arabinose, and galactose.

The salt requirement of each isolate was determined by using T_1N_1 agar and the same medium without NaCl (T_1N_0).

Determination of cytochrome oxidase was made by the filter paper method of Kovacs [32] using N,N,N'N'-tetramethyl-p-phenylenediamine dihydrochloride.

Production of B-D-galactosidase was induced by growing cultures on T_1N_1 agar with 0.1% lactose added. The B-D-galactosidase was then detected by the toluene modification method of o-nitrophenyl-B-D-galactopyranoside (ONPG) hydrolysis described by Paik [33].

Fermentation of sucrose, arabinose, and galactose was determined by spotting cultures onto plates containing sucrose

and galactose media described by Watkins et al. [51], as well as the same media with arabinose substituted for galactose. Acid production on any of these media was recorded as a positive reaction.

Based on these six characterization tests, isolates were divided into 5 groups; presumptive V. cholerae, presumptive V. mimicus, presumptive V. parahaemolyticus, presumptive V. vulnificus, and other Vibrio-like organisms. These Vibrio-like cultures included all those giving biochemical reactions that did not fit the biochemical patterns of the 4 Vibrios of interest in this study. Repesentative numbers of these cultures were tested serologically as described below and by the API 20E system to verify that they were not strains of the species listed above. No further attempts were made to identify these Vibrio-like cultures, but they have been retained in the culture collection.

Representative cultures from each of the 5 groups were further screened by serological testing with anti-flagellar (anti-H) antibody. Anti-H coagglutination reagents for V. vulnificus, V. parahaemolyticus, V. cholerae, and V. mimicus were obtained from J. Simonson and R. J. Siebeling of Louisiana State University. The slide agglutination test using TET buffer suspensions as described by Simonson et al. [41] was used.

Identification of representative isolates was also verified by the API 20E system. Results were reported as the highest dilution in which each Vibrio species was found. All cultures

have been maintained on long term preservation medium [17] for possible pathogenicity testing at a later date.

Impediments Encountered in Conducting the Project:

To relate the results of this project to commercial operations, the investigators felt it was necessary that all harvesting and shipping be carried out as near as possible to normal commercial practices. This dictated that oyster samples be harvested from what ever location was being harvested on the date sampling was scheduled. Harvesting from specific areas was further compounded by weather conditions and seasonal closures of shellfish harvesting areas. Therefore it was not possible to obtain all the oyster samples initially planned or to obtain them on a regular basis from high or low salinity areas. Further, shipment of the oysters had to be coordinated with the processing plants. On several occasions transport trucks did not arrive at the dock to pick up the oysters from the boat, and shipment under desirable commercial conditions could not be maintained.

A second problem which plagued the project was an invasion of the laboratory by fruit flies. The ventilation system in the multipurpose building was not adequate to prevent these insects from entering the laboratory from other parts of the building. Further, the aroma produced by the incubating oysters and media attracted the flies. These small insects were capable of crawling into closed petri dishes where they walked through developing colonies causing cross contamination. Frequently they laid eggs

in the plates and the larva crawled over plates spreading contamination. These invasions resulted in a loss of numerous cultures which compromised the validity of the vibrio data from several of the studies conducted in 1985. Culture handling techniques had to be redesigned to overcome this problem.

A third difficulty was the large number of cultures of bacteria that were isolated from the oysters. These included 1088 fecal coliform and 3984 vibrio and Aeromonas cultures. We had expected to deal with only about half this number of cultures. Therefore, the additional tests necessary to tentatively identify the culture and the time necessary to maintain a culture collection of this size put a severe strain on the project.

RESULTS

Commercial Handling Studies:

A synopsis of harvest area data and transport time-temperature data is presented in table 1. All of the harvest locations were classified as approved shellfish harvesting areas. With one exception (8-21-85), the mean of fecal coliform counts performed on duplicate water samples collected at each area at the time of oyster harvesting was below 14.

The oysters internal temperature at the time of harvest paralleled the water temperature and ranged from 13 to 29°C. When the oysters reached the dock their internal temperature was within 3°C of the harvest temperature and was typically 2°C cooler than at harvest. The lowering of temperature was attributed to evaporation of water from the shell which cooled the oysters. In most instances the temperature of the oysters increased 1 to 2°C after being loaded into the truck and before cooling began. At no time did the oysters exceed a temperature of 29°C.

The average time between harvest and the start of processing at the plant was 19.5 hours with the average length of time that the oysters were on the deck of the boat after harvest being 7.9 hours. The oysters typically remained above 15°C for 16.2 hours after harvest and rarely reached 10°C.

A summary of the commercial handling bacteriological data, presented in table 2, shows an increase in the fecal coliform and aerobic plate counts at each sampling point after harvest. It is

Table 1. Synopsis of harvest area and transport time-temperature data from commercial handling studies.

Date	Harvest Location	Harvest Area Conditions				Oyster Temperature (°C)				Time Factor (hours)				
		Water Salinity (ppt.)	Air Temp (°C)	Water Temp. (°C)	Fecal coliform (MPN)	at Harvest (a)	at Dock (b)	Harv. to Plant Max. (c)	Harv. to Plant Min. (c)	H-D (d)	D-P (e)	Total (f)	>15°C (g)	>10°C (h)
03-26-85	Taylors Bayou	7.5	19	19.5	4.5	20	22	22	10	3.5	14.5	18	9.5	16
04-25-85	Billet Bayou	16	23	24	4.5	23	21.5	26	13	8	14	22	18	22
05-21-85	Hellhole Bayou	10	24	26	4.5	24	21	26	11	12	10	22	21	22
06-20-85	Hellhole Bayou	7	21	24	4.5	24	23	26	16	12	10	22	22	22
07-18-85*	Bayou Cheland	27	27	26	9.3	27	25	29	5	10	11	21	15	16
08-21-85	Taylors Bayou	15	26	29	17	29	27	29	20	8.5	12	20.5	20.5	20.5
10-03-85	Bay Junop	18	18	21	11	20	20.5	20.5	13	6	12	18	16	18
12-04-85*	Grand Bay DuLarge	16	15	13	4	13	--	--	--	--	--	--	--	--
03-18-86	Grand Bay DuLarge	23	23	21	<1.8	21	21.5	24	12	6	12	18	12	18
04-28-86*	4-League Bay	23	23	21	4.5	22	--	23	5	4	14	18	13	14
07-01-86	Oyster Bayou	7	29	27	7.8	27	28	29	12	9	9	18	16	18
08-08-86	Oyster Bayou	9	26	27	7.8	27	24	27	19	9	9	18	18	18
10-07-86	Oyster Bayou	26	21.5	24	6.8	24	22	24	10	7	11	18	13.5	17.5

a - Internal temperature of oysters when harvested.
b - Internal temperature of oysters when landed at dock.
c - Maximum and minimum temperature to which oysters exposed (between harvest and the processing plant).
d - Time between harvest and dock.
e - Time between dock and when truck was unsealed at plant.
f - Total time after harvest.
g - Hours after harvest oysters exposed to temperature > 15°C.
h - Hours after harvest oysters exposed to temperature > 10°C.
* - Oysters not transported in refrigerated truck.

Table 2. Level of bacteria in shellstock oysters taken at several points during commercial operation. Duplicate samples were analyzed from each lot of oysters.

	Harvest	Dock	Plant
Number of Lots	13	12	12
Fecal Coliforms			
Range (MPN/100g)	<30-1,700	<30-4,300	<30-110,000
Geometric Mean (MPN/100g)	74	180	550
Samples with MPN >230/100g	7.7%	37.5%	45.8%
Lots with MPN >230/100g	16.6%	50.0%	70.0%
E. coli			
Range (MPN/100g)	<30-91	<30-4,300	<30-1,500
Geometric Mean (MPN/100g)	34	67	65
Samples with MPN >230/100g	0%	12.5%	12.5%
Lots with MPN >230/100g	0%	25%	25%
Aerobic Plate Count			
Range (CFU/g)	<300-530,000	580-290,000	1,200-310,000
Geometric Mean (CFU/g)	2,500	6,000	12,000
Samples with >500,000 CFU/g	3.8%	0%	0%
Lots with >500,000 CFU/g	3.8%	0%	0%

significant that 70% of the lots of oysters followed in this study had at least one sample that exceeded a fecal coliform MPN of 230/100g when they reached the processing plant. While there was an increase in E. coli after harvest, the majority of the increase occurred while the oysters were still on the boat, and only 25% of the lots had E. coli counts >230/100g.

There was a concern that increases in the E. coli counts, as detected by the APHA IMViC procedure, may have been masked by the large increase in other fecal coliforms. In one study, we employed the MUG procedure [31] for measuring E. coli and found that both procedures gave comparable counts even when the fecal coliform levels increased.

Fecal coliform cultures were identified in an effort to determine the types of fecal coliforms in addition to E. coli that were multiplying in the oysters. Klebsiella, Enterobacter, and Citrobacter species were encountered in oysters at the time of harvest, and all species were seen to increase in oysters after harvest. Klebsiella usually increased the most, but, in some samples, Enterobacter predominated. Citrobacter were encountered only occasionally and rarely dominated.

As shown in table 3, there was a tendency for fecal coliform counts to be higher at harvest and to increase more in the summer months, but increases did occur in other months, 10-85 and 3-86. Large increases in number of E. coli were encountered only during the summer months, 6-85, 7-85, and 7-86. However, correlation analysis did not support a significant relationship

Table 3. Bacteria in oyster samples collected from points in the commercial transport. Numbers are geometric mean of duplicated samples rounded to two significant figures. Less than (<) values result from one of the duplicate values being indeterminate (<30 or <300).

Sample Date (Mo - Yr)	Fecal Coliform (MPN/100g)			*E. coli* (MPN/100g)			Aerobic Plate Count (CFU/g)		
	Harvest	Dock	Plant	Harvest	Dock	Plant	Harvest	Dock	Plant
3-85	<30	55	<60	<30	33	<60	<330	1,600	6,000
4-85	<30	<60	630	<30	<30	<30	<490	3,000	26,000
5-85	91	250	200	57	51	<52	13,000	100,000	170,000
6-85	630	2,400	20,000	30	260	550	15,000	21,000	300,000
7-85	220	1,400	6,000	<33	990	330	4,000	5,200	9,000
8-85	36	600	51	<33	110	<30	2,500	9,000	9,100
10-85	83	200	530	<30	<83	<83	1,400	2,800	1,900
12-85	<33	NS	NS	<33	NS	NS	80,000	NS	NS
3-86	<33	<67	6,500	<30	52	<30	830	2,900	6,300
4-86	<30	33	<30	<30	33	<30	510	1,300	2,300
7-86	<47	83	800	<47	<33	120	4,000	6,400	18,000
8-86	460	250	4,700	<33	<33	<30	5,000	4,500	12,000
10-86	91	91	51	<33	57	51	380	1,400	3,600

NS - Not Sampled.

between either fecal coliform or E. coli counts and the water temperature at the time of harvest.

Aerobic plate counts were higher in the oysters harvested in the summer months with the exception of the 12-85 sample. One of the duplicate samples from that month had an APC of 530,000 CFU/g. It is possible that one of the oysters in that sample was dead leading to the high count. With the above exception, no other oyster sample in the commercial handling study exceeded the 500,000 APC level. It should be noted that 4 of the 5 samples with APC counts >10,000 CFU/g at the plant were harvested in areas with salinities of 10 ppt or less and were harvested between May and August. This suggests that the low salt levels in the oysters may allow for a more rapid multiplication of bacteria.

Salmonella were not isolated from any of the oyster samples taken at harvest or at the processing plant. Failure to recover Salmonella from these oyster samples indicated the incidence in oysters from approved areas is low.

In this study we had anticipated enumerating the vibrios by the three-tube MPN procedure. Our results were such that we frequently failed to isolate specific vibrios from lower dilution MPN tubes when we successfully isolated them from higher dilution tubes. Reasons for isolation failure were thought to include: 1) The use of a single non-species selective Vibrio enrichment technique may have allowed species present in the largest concentrations to dominate, thus obscuring organisms present in

lower numbers. 2) Some species may have been overlooked in the selection of Vibrio-like colonies from TCBS isolation plates. As a result of these limitations, the authors felt that assigning MPN values to each Vibrio species would be misleading. Therefore, results were reported as the highest dilution from which isolation of each species could be adequately confirmed.

Levels of vibrios and Aeromonas hydrophila were determined at the time of harvest and when the shellstock oysters were taken off of the truck at the plant the following morning. Due to the problems mentioned previously (fruit flies and lack of refrigerated transport trucks), data from only six of the sampling periods is presented in table 4.

Vibrio vulnificus, V. parahaemolyticus and Aeromonas hydrophila were present during all sampling periods and showed increases of 1 to 4 orders of magnitude by the time the oysters reached the plant. V. cholerae was detected in only two of the commercially handled samples at harvest, and, in both cases, it increased by 1 order of magnitude during commercial handling. V. mimicus was isolated sporadically and it is difficult to determine if multiplication occurred with this organism.

Results clearly indicate that some of these bacteria of human health concern are capable of multiplying in shellstock oysters under currently used commercial practices.

Storage Studies:

Storage studies were undertaken to determine how bacterial

Table 4. Levels of Vibrio species and Aeromonas hydrophila in shellstock oysters at harvest (H) and when they were received at the processing plant (P). Numbers are the greatest dilution of oyster sample from which the species was isolated. Blanks indicate that the organism was not isolated from a 1 gram sample.

Date	V. cholerae		V. mimicus		V. parahaemolyticus		V. vulnificus		A. hydrophila	
	H	P	H	P	H	P	H	P	H	P
8-85					10^{-1}	10^{-3}		10^{-2}	10^{-1}	10^{-2}
10-85			10^{-2}		10^{-3}	10^{-2}	10^{-3}		10^{0}	10^{-3}
3-86					10^{-2}	10^{-3}	10^{-1}	10^{-4}	10^{-1}	10^{-3}
7-86	10^{-2}	10^{-3}			10^{-1}	10^{-5}	10^{-3}	10^{-5}	10^{0}	
8-86	10^{-1}	10^{-2}		10^{0}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}
10-86					10^{-1}	10^{-3}		10^{-3}		10^{-3}

populations in oysters might respond over time to specific temperatures.

The storage studies confirmed that fecal coliforms including E. coli do not increase in oysters held at 10°C for up to 5 days, and the APC increases only slightly during that period (table 5.) (NOTE: The day 1, 10°C counts are the same as the harvest counts since the oysters were held at a temperature of <10°C following harvest and while being returned to the laboratory for analysis which was performed approximately 1 day after harvest.)

At a constant storage temperature of 22°C, significant increases in the indicator bacteria were noted within 1 day, and the counts further increased at 3 days. While the APC showed additional increases at 5 days, the fecal coliform and E. coli counts were often lower. These decreases, noted in tables 6 and 7, were attributed to the overgrowth of the indicator bacteria by other microorganisms.

A storage temperature of 30°C sharply increased the APC (table 5) but did not always result in a higher fecal coliform count than seen at 22°C (table 6). In most cases, the E. coli counts in oysters stored for one day at 30°C were greater than in oysters stored at 22°C suggesting that higher storage temperatures favor E. coli multiplication.

The types of fecal coliforms other than E. coli which developed in the oysters in the storage studies were similar to those in the commercial handling studies. No species was favored by any of the temperatures, but Klebsiella generally dominated.

Table 5. Levels of bacteria in shellstock oysters stored at different temperatures. Result from 13 lots of oysters. Duplicate samples were analyzed on day 1 oysters stored at <10°C. One sample was analyzed from each lot of oysters on other days and temperatures.

	Days stored at 10°C			Days stored at 22°C			Days stored at 30°C	
	1*	3	5	1	3	5	1	3
Number of Samples Examined	26	13	13	13	13	13	13	11
Fecal Coliforms								
Geometric Mean (MPN/100g)	74	56	54	260	360	210	140	540
Samples with MPN >230/100g	7.7%	7.7%	7.7%	38.5%	46.2%	25%	30.8%	38.5%
E. coli								
Geometric Mean (MPN/100g)	34	35	32	47	110	56	64	220
Samples with MPN >230/100g	0%	0%	0%	0%	15.4%	8.3%	15.4%	30.8%
Aerobic Plate Count								
Geometric Mean (CFU/g)	2,500	2,900	4,000	7,600	30,000	380,000	22,000	300,000
Samples with >500,000 CFU/g	3.8%	0%	0%	0%	15.4%	38.5%	7.7%	38.5%

* 10°C, day 1 counts are same as harvest counts.

Table 6. Fecal coliform counts (MPN/100g) in oysters stored at various temperatures.

DATE	10° C			22° C			30° C	
(MO-YR)	1 DAY*	3 DAY	5 DAY	1 DAY	3 DAY	5 DAY	1 DAY	3 DAY
3-85	<30	<30	<30	91	<30	---**	210	4,300
4-85	<30	<30	<30	230	230	9300	<30	110
5-85	91	36	<30	430	9,300	73	91	200
6-85	630	78	560	13,000	220,000	110,000	64,000	360,000
7-85	220	430	230	4,300	2,400	930	430	4,300
8-85	36	<30	61	36	150	110	<30	<30
10-85	83	150	91	4,300	2,400	110	430	---
12-85	<33	<30	<30	<30	<30	36	36	36
3-86	<33	<30	36	<30	<30	<30	36	<30
4-86	<30	200	<30	<30	<30	<30	<30	---
7-86	<47	<30	<30	36	430	91	36	2,400
8-86	460	91	91	2,400	430	36	930	930
10-86	91	36	<30	150	73	230	<30	230

* - 10°C, day 1 counts are same as harvest counts.

** - Not sampled.

Table 7. *E. coli* counts (MPN/100g) in oysters stored at various temperatures.

DATE	10° C			22° C			30° C	
(MO-YR)	1 DAY*	3 DAY	5 DAY	1 DAY	3 DAY	5 DAY	1 DAY	3 DAY
3-85	<30	<30	<30	91	<30	---**	210	4,300
4-85	<30	<30	<30	<30	<30	<30	<30	73
5-85	57	36	<30	150	9,300	36	91	91
6-85	30	<30	<30	180	22,000	1,200	430	15,000
7-85	<33	36	91	73	150	91	430	4,300
8-85	<33	<30	30	<30	30	30	<30	<30
10-85	30	<30	<30	<30	230	36	430	---
12-85	<33	<30	<30	<30	<30	36	36	36
3-86	<30	<30	<30	<30	<30	<30	<30	<30
4-86	<30	200	<30	<30	<30	<30	<30	---
7-86	<47	<30	<30	36	230	91	36	2,400
8-86	<33	36	<30	30	<30	<30	36	<30
10-86	<33	<30	<30	<30	<30	91	<30	91

* - 10°C, day 1 counts are same as harvest counts.

** - Not sampled.

Salmonella was isolated from one lot of oysters (3-85) during the storage studies. Isolates were found in the stored samples; 3 days at 10°C, 5 days at 10°C, and 1 day at 30°C. Failure to recover Salmonella at other times and temperatures suggested that the organism was not reproducing.

Tables 8 through 12 show levels of vibrios and Aeromonas hydrophila in shellstock oysters stored under controlled temperatures. As indicated in table 8, V. cholerae was present in detectable levels at harvest (day 1, 10°C) in three lots (4-86, 7-86, & 8-86) and detected sporadically in other lots during storage. Levels of V. cholerae in the 8-86 lot of oysters stored at 10°C may be interpreted as increasing. However, this could be a result of uneven accumulation of bacteria in the oysters. During the other months, V. cholerae levels remained constant for 3 days and then dropped to undetectable levels by day 5. At 22°C and 30°C V. cholerae showed increases above the harvest level, but no clear pattern of increase was seen. V. cholerae were isolated predominately during the summer months and levels generally increased when harvest water temperatures were high and salinities were low.

V. mimicus was encountered sporadically, but in one lot of oysters (7-86) increased at all three storage temperatures (table 9). The salinity in the harvest area, when the 7-86 lot of oysters was harvested, was low and may have encouraged the increase of this organism in this sample.

Table 8. Greatest dilution of oyster sample from which Vibrio cholerae was isolated. Blanks indicate the organism was not isolated from a 1g sample.

Date Harvested	Days stored at 10°C			Days stored at 22°C			Days stored at 30°C	
	1*	3	5	1	3	5	1	3
8-85		10^{-4}						
10-85				10^{-2}				---**
12-85								
3-86								
4-86	10^{-1}	10^{-1}		10^{-2}				---
7-86	10^{-2}	10^{-2}		10^{-2}	10^{-5}	10^{-2}		10^{-3}
8-86	10^{-1}	10^{-5}		10^{-4}		10^{-3}	10^{-3}	10^{-2}
10-86								

* - 10°C, day 1 levels are same as harvest levels.

** - Not sampled

Table 9. Greatest dilution of oyster sample from which Vibrio mimicus was isolated. Blanks indicate the organism was not isolated from a 1g sample.

Date Harvested	Days stored at 10°C			Days stored at 22°C			Days stored at 30°C	
	1*	3	5	1	3	5	1	3
8-85								--**
10-85	10^{-2}							
12-85	10^{-1}							
3-86								
4-86		10^{-2}						--
7-86		10^{0}	10^{-2}		10^{-4}	10^{-4}	10^{0}	10^{-4}
8-86					10^{-2}			
10-86		10^{0}						

* - 10°C, day 1 levels are same as harvest levels.

** - No sample.

Table 10. Greatest dilution of oyster sample from which Vibrio parahaemolyticus was isolated. Blanks indicate the organism was not isolated from a 1g sample.

Date Harvested	Days stored at 10°C			Days stored at 22°C			Days stored at 30°C	
	1*	3	5	1	3	5	1	3
8-85	10^{-1}	10^{-1}	10^{-1}	10^{-3}	10^{-4}	10^{-4}	10^{-3}	10^{-4}
10-85	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-4}	10^{-4}	10^{-3}	--**
12-85	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-3}	10^{-4}
3-86	10^{-2}	10^{-2}	10^{-1}	10^{-4}	10^{-4}	10^{-4}	10^{-5}	10^{-5}
4-86	10^{-4}	10^{-4}	10^{-5}	10^{-4}	10^{-4}	10^{-5}	10^{-5}	--
7-86	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-5}	10^{-3}	10^{-5}	10^{-1}
8-86	10^{-2}	10^{-3}	10^{-2}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-5}
10-86	10^{-1}	10^{-1}	10^{-1}	10^{-3}	10^{-5}	10^{-4}	10^{-5}	10^{-5}

* - 10°C, day 1 levels are same as harvest levels.

** - Not sampled.

Table 11. Greatest dilution of oyster sample from which Vibrio vulnificus was isolated. Blanks indicate the organism was not isolated from a 1g sample.

Date Harvested	Days stored at 10°C			Days stored at 22°C			Days stored at 30°C	
	1*	3	5	1	3	5	1	3
8-85		10^{-3}	10^{-1}	10^{-2}	10^{-3}			10^{-2}
10-85	10^{-3}	10^{-1}	10^{-3}	10^{-3}	10^{-4}	10^{-3}	10^{-2}	---**
12-85			10^{-3}	10^{-3}	10^{-1}	10^{-2}	10^{-2}	10^{-4}
3-86	10^{-1}	10^{-1}	10^{-1}	10^{-2}	10^{-3}	10^{-3}	10^{-4}	10^{-3}
4-86	10^{-5}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-5}	---
7-86	10^{-3}	10^{-2}	10^{-2}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-3}
8-86	10^{-4}	10^{-4}	10^{-4}	10^{-5}	10^{-3}	10^{-2}	10^{-5}	10^{-4}
10-86				10^{-2}	10^{-2}			10^{-4}

* - 10°C, day 1 levels are same as harvest levels.

** - Not sampled.

Table 12. Greatest dilution of oyster sample from which Aeromonas hydrophilia was isolated. Blanks indicate the organism was not isolated from a 1g sample.

Date Harvested	Days stored at 10°C			Days stored at 22°C			Days stored at 30°C	
	1*	3	5	1	3	5	1	3
8-85	10^{-1}	10^{-0}	10^{-3}	10^{-3}	10^{-4}		10^{-2}	10^{-2}
10-85	10^{0}	10^{-1}	10^{0}	10^{-3}	10^{-4}	10^{-4}	10^{-3}	---**
12-85	10^{-2}	10^{-1}	10^{-1}	10^{-2}	10^{-4}	10^{-1}	10^{-3}	10^{-3}
3-86	10^{-1}	10^{-2}	10^{-2}	10^{-1}	10^{-4}	10^{-5}	10^{-5}	10^{-5}
4-86	10^{-1}	10^{-2}	10^{-5}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	---
7-86	10^{0}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-5}	10^{-2}	10^{-4}
8-86	10^{-2}	10^{-3}	10^{-4}	10^{-3}	10^{-3}	10^{-5}		10^{-5}
10-86		10^{-2}	10^{-4}	10^{-3}		10^{-5}	10^{-5}	10^{-5}

* - 10°C, day 1 levels are same as harvest levels.

** - Not sampled.

As indicated in table 10, V. parahaemolyticus was isolated from all eight lots and showed increases at temperatures above 10^0C. After only 1 day of storage, levels of V. parahaemolyticus at 22^0C and 30^0C were 2 to 3 orders of magnitude higher than those at harvest. These levels in most cases continued to increase during storage with the higher temperature usually having a greater number or bacteria. This indicates a direct relationship between multiplication of V. parahaemolyticus and storage temperatures.

Vibrio vulnificus levels remained stable or decreased during storage at 10^oC. After 1 day of storage at the higher temperatures, counts increased one to two orders of magnitude above the harvest level. Prolonged incubation resulted in a slight increase in numbers followed by a decrease (table 11).

V. parahaemolyticus and V. vulnificus were present at unexpected high levels in the 4-86 lot of oysters. Fecal coliform and aerobic plate count data do not provide any indication of unusual pollution levels or of abuse in handling the oysters. Environmental changes may have caused an unexpected bloom of these organisms during this sampling time.

Aeromonas hydrophila levels increased in most lots at all storage temperatures (table 12) and levels generally continued to increase with storage time.

These studies indicate that storing shellstock oysters below 10^0C will prevent significant multiplication of V. vulnificus, V. parahaemolyticus and possibly V. mimicus. The question of V.

cholerae multiplication at 10°C remains unresolved. Although A. hydrophila reproduced at 10°C, its rate of multiplication at this temperature was considerably less than at higher temperatures.

Oysters from the same lot were used in both the storage and commercial handling studies. Further, bacteriological analyses were run on oysters from both studies approximately 1 day after harvest. This allowed for an evaluation of the effect of storage temperature as well as handling procedure on fecal coliform counts. Correlation analysis were run between the fecal coliform counts in the oysters taken at the plant (commercial handling studies) and counts after one day of storage at 22°C and at 30°C (storage studies). Similar correlations were made with E. coli counts from both studies. All correlations were significant at the 0.01% level. The correlation values with the 30°C storage temperature were higher than with the other storage temperature. This is to be expected since most of the oysters in the commercial handling studies were exposed to temperatures several degrees above 22°C during storage.

The mean APC from the plant samples (commercial handling study, table 2) fell between the mean APC levels for the 22°C and 30°C day 1 samples (storage studies, table 5). E. coli levels in the commercially shipped oysters closely paralleled the 30°C storage data. Mean fecal coliform counts in the commercially shipped oysters were higher, and the number of samples that exceeded 230 MPN per 100g was greater than the numbers at either

storage temperature. This may indicate that factors [37] such as contaminated sacks, rough handling or high humidity in the transport trucks, not encountered in the storage studies, may have played a role in the increased number of fecal coliforms in oysters shipped through normal commercial channels.

Because of the nature of the data, it was not appropriate to correlate fecal coliform counts and levels of vibrios in the oysters. Visual inspection of the data did not reveal any relationship between change in fecal coliform or E. coli counts and levels of vibrios or A. hydrophila.

The ability of fecal coliforms to grow in the shell liquid from oysters was established by the following experiment. Oysters were harvested from Davis Bay off the GCRL pier and brought into the laboratory. A bay water sample was collected at the same time. The oysters were held at 25°C for 4 hours allowing time for metabolites excreted from the oyster to accumulate in the shell liquid. Six oysters were sacrificed and the shell liquid collected. Fecal coliform counts were made on the bay water and the shell liquid. The bay water, shell liquid (in a test tube) and six oysters were held for approximately 20 hours at 25°C. Shell liquid was then collected from the incubated oysters and analyzed along with the bay water and the incubated shell liquid for fecal coliforms. The results of five such experiments are presented in table 13. Fecal coliforms were found to multiply in the shell liquid at about the same rate regardless of whether the liquid was in the oyster or in the test tube. Failure of the

Table 13. Fecal coliform counts (MPN/100 ml) in oyster shell liquid and in bay water before and after incubation at 25°C for 20 hours.

BAY WATER SALINITY (ppt)	BAY WATER		SHELL LIQUID FROM OYSTERS		SHELL LIQUID INCUBATED IN TEST TUBE	
	0 HR	20 HR	0 HR	20 HR	0 HR	20 HR
10	240	170	--	--	18	24000
14	68	20	230	1700	230	1700
15	<20	20	<20	1300	<20	490
18	40	<20	78	1700	78	1300
25	33	33	78	790	78	490

fecal coliforms to increase in the bay water indicated that organic matter from the oyster provided the nutrients necessary to permit the fecal coliforms to reproduce.

DISCUSSION

Oysters are filter feeders and can concentrate bacteria from the waters in which they live. The concentration factor depends on the pumping rate of the oyster as well as environmental conditions including temperature, salinity, turbidity and the type and quantity of food. Within a population of oysters, individuals pump at different rates leading to an uneven distribution of bacteria among members of the population. As oysters are disturbed during harvest, they close their shells and trap some of the estuarine water along with the associated bacteria. Therefore, the microflora within the oyster shell at the time of harvest will, in part, represent the bacteria in the environment from which it was harvested, but all oysters may not contain the same numbers of bacteria.

It is typical for estuarine waters approved for shellfish harvesting to contain fecal coliforms including Escherichia coli. These indicator bacteria are usually low in number and may be below a detectable level (<2/100 ml). Estuarine waters are also the natural habitat for vibrios including Vibrio cholerae, Vibrio parahaemolyticus, Vibrio mimicus and Vibrio vulnificus, some strains of which may be pathogenic. Aeromonas hydrophilia is an aquatic bacterium, now recognized as a primary pathogen and frequently found in estuarine waters. Therefore, it is not unexpected to find that oysters, at the time of harvest from approved shellfishing waters, contain the bacteria listed above. The number of each species of bacteria found in the oyster is

dependent upon both their number in the water and the concentration factor of the oyster.

The fate of specific bacteria in shellstock oysters depends upon a variety of factors. Since temperature is a controlling factor in the growth of all bacteria, the lower the storage temperature, the less chance that the bacteria will reproduce in oysters. Some estuarine vibrios are known to be adversely affected by low temperatures and may not survive at temperatures below 10°C [21].

If the temperature is high enough to permit growth of the bacteria, the availability of utilizable organic matter and oxygen become controlling factors. The fecal coliforms, A. hydrophilia and vibrios are capable of fermentative metabolism, therefore, oxygen availability should not be a limiting factor. The quality and quantity of organic matter in estuarine waters is usually not sufficient to support the growth of fecal coliforms, but it is probably adequate for the autochthonous estuarine bacteria. However, the estuarine water trapped within the oysters shell becomes enriched with organic metabolites from the oyster and can support growth of indicator bacteria.

Another factor which affects the fate of a specific species of bacteria in oysters is the competition from other microorganisms which may be present. These competitive microorganisms may prevent growth of certain bacteria by utilizing the available food or by secreting products which are inhibitory or toxic to the bacteria. In nature, biological

successions are typical and may be the cause of increases and decreases of particular bacteria noted in this study.

Of the factors mentioned above, temperature is the only one that can be controlled by man. Therefore, it was important to evaluate the effect of temperature on the fate of fecal coliforms, A. hydrophilia and vibrios in shellstock oysters.

The techniques used to harvest and transport oysters in this study were typical of those used throughout the commercial oyster industry in Louisiana. Therefore, the bacteriological findings in the commercial handling portion of this study should be representative of the industry throughout Louisiana and possibly, the entire Gulf coast oyster industry.

This study has verified previous reports [3, 4] showing that oyster harvesting and transport procedures typically used in the industry provide conditions which permit fecal coliform bacteria to multiply. Verification that fecal coliforms can multiply in shellstock oysters emphasizes that levels of these bacteria in post-harvest oysters should not be used as an indicator of the water quality in the area from which the oysters were harvested [4].

E. coli increases did not always accompany fecal coliform increases in the commercial handling studies. E. coli was found to be dominant only when fecal coliform counts were low. This suggests that fecal coliforms other than E. coli are more competitive and reproduce faster in the oysters. On two occasions we did observe E. coli counts to increase from <36 to >230/100g

during a 12 hour period while the oysters were on the deck of the boat. The temperature during this period was 27°C or less. Higher E. coli levels were reached as these oysters were being transported to the plant.

The storage studies have shown that temperature is a controlling factor in the multiplication of indicator bacteria in oysters. Indicator bacteria did not increase in oysters held at 10°C for five days. At temperatures of 22 and 30°C multiplication of fecal coliforms including E. coli occurred in some but not all lots of oysters. In all instances when the indicator bacteria did not multiply, their levels in the oysters at the time of harvest were low or below the detectable level of 30/100g (tables 3 and 4). The higher temperature seemed to favor the multiplication of E. coli.

The dominant species of fecal coliform which multiplied in oysters was Klebsiella. Some Klebsiella pneumonia strains have been reported to be enteropathogenic, so its presence in large numbers may be of concern. Boutin et al. [8] tested the pathogenicity of Klebsiella strains isolated from oysters and concluded that they were not a public health risk. However, these researchers expressed concern about the levels of Klebsiella found in some oysters. Under the worse abuse conditions of our storage studies (3 days at 30°C), Klebsiella only reached levels of 10^4/g which was 5 orders of magnitude less than the number of cells necessary to produce a significant pathogenic response in adult mice.

Vibrio cholerae, V. parahaemolyticus, V. vulnificus, and A. hydrophila were found in shellstock oysters at harvest. The levels of these organisms depended upon factors previously described. All of these species increased in number in the oysters after harvest, with the increases being temperature mediated. Vibrio parahaemolyticus and V. vulnificus did not multiply in oysters stored at 10^0C while A. hydrophila did.

It is important to point out that several factors are involved which determine if individuals will become ill by consuming a particular bacteria in a food. First, the strain of the bacteria being ingested must be pathogenic. Second, even if the strain ingested is pathogenic, an infective dose of the organism must be consumed. The infective dose of some vibrios is quite large, but infective dose levels may very considerably among strains of the same organism. Our knowledge of infective dose levels of all vibrios is limited. Third, there are physiological and immuniological differences among individuals that gauge how they may react to specific pathogens or dose levels. In this study, we have not attempted to measure the pathogenicity of the strains isolated but have centered study on the levels of potential pathogens that may develop in the oysters under specific conditions.

Studies have indicated that consumption of 10^6-10^9 CFU of pathogenic strains of V. parahaemolyticus may cause gastroenteritis in man [49]. In several lots of oysters stored above 10^0C, V. parahaemolyticus levels reached 10^4-10^5 cells per

gram. In the 4-86 lot, V. parahaemolyticus levels at harvest were 10^4/g and increased during storage. (NOTE: 10^5 was the highest dilution run, so levels may have been even higher in some cases.) These levels are approaching the infectious dosage level of 10^6-10^9 cells. However, it is unknown if the strains present were pathogenic.

Pathogenicity of V. parahaemolyticus is generally based on the presence of Kanagawa hemolysin. Kanagawa positive strains are generally isolated from patients with gastroenteritis, while strains isolated from seafood and marine environments are generally Kanagawa negative [19]. Isolates from this study were not tested for the presence of Kanagawa hemolysin. Several studies have been conducted to develop more accurate means of determining the pathogenicity of V. parahaemolyticus and a more selective methodology for isolating pathogenic strains from the environment [16, 19, 20]. Until additional information is available concerning the pathogenicity of V. parahaemolyticus, it will be difficult to determine the significance of its presence in oysters. Because the strains of this organisms found in the environment are generally considered non-pathogenic, multiplication of this organism does not necessarily indicate a human health risk. Since levels of V. parahaemolyticus in oysters at the time of harvest are generally low, cooling the oysters as soon after harvest as practical would be a prudent measure to reduce multiplication of this organism and any health risk associated with it.

Unlike V. parahaemolyticus, where only some strains are pathogenic, 90% of environmental isolates of V. vulnificus are capable of causing disease in man [50]. Inadequate information on infectious dosages of V. vulnificus for man makes it difficult to determine the significance of this organism in oysters. Animal studies with iron treated mice have suggested that strains differ significantly in their ID_{50} (7.6 to >20,000 CFU) [30]. V. vulnificus was commonly found in the oysters at levels of 10^3 to 10^4/g at harvest and frequently increased during transport.

While most isolates are capable of causing disease and the organism is commonly found in oysters, V. vulnificus infections resulting from the consumption of oysters are uncommon because pre-disposing health factors are required for infection [6]. Perhaps the best defense against this organism is to educate the general public, especially high-risk individuals, to the dangers involved in eating raw oysters. In addition, chilling oysters to temperatures below 10^oC will help to slow multiplication of V. vulnificus.

V. cholerae serotypes 01 and non-01 have been found in aquatic environments and oysters in Maryland, Louisiana, and Florida [12, 13, 15, 22]. These studies have revealed that there is no correlation between fecal coliforms or other indicators of sewage contamination and V. cholerae. While V. cholerae serotype 01 is most commonly isolated from cholera cases, the non-01 serotype has been implicated in a number of outbreaks of shellfish related gastroenteritis and is now believed to be

potentially pathogenic [38]. The V. cholerae isolates in this study were not serotyped or tested for pathogenicity.

Unlike the vibrios discussed thus far, V. cholerae has been shown to multiply at temperatures below 10^0C [23]. Sporadic isolation of V. cholerae in our study was insufficient to confirm these findings. In two lots, V. cholerae levels were higher after 3 days of storage at 10^0C than at harvest, but no V. cholerae could be isolated after 5 days. In two other lots, no increases were noted at 10^0C. These findings may be a result of uneven accumulation of V. cholerae in the oysters rather than multiplication of the bacterium. Failure to isolate V. cholerae from any lot on day 5 suggests that the organism does not withstand prolonged storage at this temperature.

Aeromonas hydrophila has recently been accepted as a primary pathogen and is being recognized more frequently as the cause of gastroenteritis. The organism has been isolated from shellfish implicated in outbreaks of gastroenteritis although it is unknown if the organism was responsible for the outbreaks [1]. A. hydrophila is indigenous to aquatic environments and its human health significance is still under investigation.

Multiplication of A. hydrophila was observed at all three storage temperatures used in this study. Hood et al. [23] observed similar results in shellstock oysters stored for 7 days. Additional information is needed in order to determine levels of A. hydrophila necessary to produce gastroenteritis. Harvest levels of A. hydrophila were low (10^0-10^2) in all 8 lots of

shellstock oysters examined in this study. The organism increased in number throughout the storage period with the greatest increases at the highest temperatures. Therefore, storage of the oysters below 10^0C may prevent A. hydrophila from reaching infective dose levels.

While there was a tendency for vibrios, Aeromonas hydrophila, and fecal coliforms to increase between harvest and receipt at the processing plant, the change in levels of vibrios and A. hydrophila did not always parallel the change in numbers of fecal coliforms. Similar observations were noted in our storage studies and those of Hopkins [24]. From the data available it appears that oysters which increase in fecal coliform numbers also increase in levels of A. hydrophila and vibrios. However, failure of the fecal coliforms to increase in number did not necessarily indicate that A. hydrophila and vibrios remained at their harvest levels.

We believe that the levels of bacteria reached in the commercial handling studies were typical of the levels normally seen in the industry. We must, therefore, conclude those levels do not present a health risk because the incidence of diseases caused by these bacteria is low among healthy consumers of oysters. However, under conditions of temperature abuse in the storage studies, we did observe that some of these bacteria reached numbers near the infective dose level for pathogenic strains of some bacteria.

The NSSP Manual of Operations [5] states "Shellstock shall be shipped and stored at such temperatures and under such conditions as are necessary to minimize the potential for microbial growth and product deterioration ..." No specific storage temperatures are required while oysters are on the harvest boats. Indeed, it would be difficult to instate such a requirement because oyster harvesting boats are usually of such size as to make it impractical to equip them with refrigeration equipment. However, data presented here and elsewhere [4] have documented fecal coliform and sometimes E. coli multiplication while oysters were on the boat. In this study we did not evaluate Vibrio and Aeromonas multiplication during that portion of shipment but we believe that those bacteria could increase during that phase of transport at ambient summer temperatures. Oyster harvesters should recognize this problem and restrict harvest to the cooler times of the year or reduce the time the oysters remain on the deck of the harvest boats during the summer.

The NSSP Manual of Operations [5] has set conditions for interstate land transport of shellstock. Among these is that the conveyance be mechanically refrigerated and maintained at or below 45°F. The transport trucks used in this study did not always meet that requirement and we did observe increases in fecal coliforms and sometimes E. coli during the truck transport of the oysters. We did not develope data on the Vibrio and Aeromonas multiplication separately on the truck transport portion in the commercial harvesting studies, but we believe some

increases in these bacteria could have occurred at the temperatures under which the oysters were transported. These findings verify that the transport temperature requirement is necessary to control of bacteria growth. Shellstock transporters should recognize this fact and act responsibly.

SUMMARY

In summary we wish to address the questions asked in the introduction of this report:

(1) What storage conditions permit fecal coliform bacteria to multiply in shellstock oysters?

We have verified that a storage temperature of 10°C prevents fecal coliform bacteria from reproducing in oysters. At temperatures of 22°C and above, fecal coliforms may reproduce, but, temperature and time did not appear to be the only factor which controlled their reproduction. The normal commercial practices used in handling oysters permit fecal coliform bacteria to multiply in oysters.

(2) What types of fecal coliform bacteria multiply in shellstock oysters under various conditions?

In oysters which showed fecal coliform multiplication Klebsiella sp. were usually the dominant fecal coliform type present regardless of the storage temperature or length of storage. Escherichia coli did increase in numbers in some lots of oysters and the higher storage temperature, 30°C, favored its multiplication.

(3) Do conditions which permit fecal coliform bacteria to multiply in shellstock oysters also permit vibrios and other potentially pathogenic bacteria to multiply?

It was observed that Vibrio parahaemoliticus, Vibrio vulnificus, Vibrio cholerae, Vibrio mimicus, and Aeromonas hydrophlia multiplied in oysters under normal commercial transport and in oysters stored at 22°C and 30°C. Only A.

hydrophila was capable of multiplying at a temperature of 10°C; a temperature at which the fecal coliforms could not multiply.

(4) Is there a correlation between fecal coliform or E. coli multiplication and vibrio or other pathogen multiplication?

It was observed that the vibrios and A. hydrophila increased in number in the same samples in which the fecal coliforms increased, but these potential pathogens increased in some samples when the fecal coliforms failed to multiply. It thus appears that no correlation exists between the multiplication of the fecal coliforms and the potential pathogens studied in shellstock oysters.

LITERATURE CITED

1. Abeyta, C., Jr., C. A. Kaysner, M. M. Wekell, J. J. Sullivan and G. N. Stelma. 1986. Recovery of Aeromonas hydrophila from oysters implicated in an outbreak of foodborne illness. Journal of Food Protection 49(8):643-646.

2. American Public Health Association. 1970. Recommended Procedures for the Examination of Seawater and Shellfish. 4th Ed., American Public Health Association, Inc. New York, NY.

3. Anonymous. 1971. The Influence of Time and Temperature on the Bacterial Quality of Shell Oysters During Processing and Shipping. Special Report, Dept. Health, Ed. and Welfare. Gulf Coast Technical Services Unit.

4. Anonymous. 1983. Bacteriological Quality of Approved Area Summer Harvested Louisiana Oysters During Harvest and Interstate Shipment. Food and Drug Administration, Shellfish Sanitation Branch, Northeast Technical Services Unit, North Kingston, R.I.

5. Anonymous. 1987 (revision). National Shellfish Sanitation Program, Manual of Operations, Part II, Sanitation of the Harvesting, Processing and Distribution of Shellfish. U. S. Dept. of Health and Human Services, Washington D. C.

6. Blake, P. A., M. H. Merson, R. E. Weaver, D. G. Hollis, and P.C. Heublein. 1979. Disease caused by a marine vibrio: Clinical characteristics and epidemiology. New England Journal of Medicine 300(1):1-5.

7. Blake, P. A., R. E. Weaver, and D.G.Hollis. 1980. Diseases of humans (other than cholera) caused by vibrios. Annual Reviews of Microbiology 34:341-367.

8. Boutin, B. K., P. L. Spaulding and R. M. Twedt. 1986. Evaluation of the enteropathogenicity of Klebsiella pneumoniae isolates from summer-harvested Louisiana oysters. Journal of Food Protection 49(6):442-444.

9. Center for Disease Control. 1977. Vibrio cholerae - Alabama. Morbidity and Mortality Weekly Report 26:159.

10. Center for Disease Control. 1979. Non-O1 Vibrio cholerae infections - Florida. Morbidity and Mortality Weekly Report 28:571-572.

11. Center for Disease Control. 1980. Cholera - Florida. Morbidity and Mortality Weekly Report 29:601.

12. Colwell, R. R., R. J. Seidler, J. Kaper, S. W. Joseph, S. Garges, H. Lackman, D. Maneval, H. Bradford, N. Roberts, E. Remmes, I. Huq and A. Huq. 1981. Occurance of Vibrio cholerae serotype-O1 in Maryland and Louisiana estraries. Applied and Environmental Microbiology 41(2):555-558.

13. Colwell, R. R. (Ed.) 1984. Vibrios in the Environment. John Wiley & Sons, New York.

14. DePaola, A., 1981. Vibrio cholerae in marine foods and environmental waters: A literature review. Journal of Food Science 46:66-70.

15. DePaola, A., M. W. Presnell, R. E. Becker, M. L. Motes, Jr., S. R. Zywno, J. F. Musselman, J. Taylor and L. Williams. 1984. Distribution of Vibrio cholerae in the Apalachicola (Florida) Bay estuary. Journal of Food Protection 47(7):549-553.

16. Dicharry, A. M. 1983. Methodology for isolation of Kanagawa positive Vibrio parahaemolyticus. Ms. Thesis, Louisiana State University.

17. Food and Drug Administration. 1978. Bacteriological Analytical Manual, 5th Ed., Association of Official Analytical Chemists, Washington, D.C.

18. Greenberg, A. E. and D. A. Hunt. 1985. Laboratory Procedures for the Examination of Seawater and Shellfish. Fifth Edition. American Public Health Association, Washington D. C.

19. Guerry, P. and R. R. Colwell. 1977. Isolation of cryptic plasmid deoxyribonucleic acid from Kanagawa - Positive strains of Vibrio parahaemolyticus. Infection and Immunity 16(1):328-334.

20. Hackney, C. R., E. G. Kleeman, B. Ray and M. L. Speck. 1980. Adherence as a method for differentiating virulent and avirulent strains of Vibrio parahaemolyticus. Applied and Environmental Microbiology 40(3):652-658.

21. Hackney, C. R., B. Ray, and M. L. Speck. 1980. Incidence of Vibrio parahaemolyticus in and the microbiological quality of seafood in North Carolina. Journal of Food Protection 43(10):769-773.

22. Hood, M. A., G. E. Ness and G. E. Rodrick. 1981. Isolation of Vibrio cholerae serotype 01 from the eastern oyster, Crassostrea virginica. Applied and Environmental Microbiology 41:559-560.

23. Hood, M. A., G. E. Ness, G. E. Rodrick, and N. J. Blake. 1983. Effects of storage on microbial loads of two commercially important shellfish species, Crassostrea virginica and Mercenaria campechiensis. Applied and Environmental Microbiology 45(4):1221-1228.

24. Hopkins, L. H. 1984. The Survival of Vibrio cholerae and Vibrio vulnificus in stored oyster shellstock. Ms. Thesis, University of Alabama in Huntsville.

25. Hosty, T. S. and C. I. McDurmont. 1974. Isolation of acid-fast organisms from milk and oysters. Health Laboratory Science 12(1):16-19.

26. Houser, L. S. 1965 (revised). National Shellfish Sanitation Program, Manual of Operations, Part I: Sanitation of Shellfish Growing Areas. U. S. Dept. Health, Ed. and Welfare, Public Health Service, Washington, D. C.

27. Johnson, J. M., S. F. Becker and L. M. McFarland. 1985. Vibrio vulnificus: Man and the sea. Journal of the American Medical Association 253(19):2850-2853.

28. Johnson, W. G., Jr., A. C. Salinger, and W. C. King. 1973. Survival of Vibrio parahaemolyticus in oyster shellstock at two different storage temperatures. Applied Microbiology 26(1):122-123.

29. Kaper, J., R. J. Seidler, H. Lockman, and R. R. Colwell. 1979. Medium for the Presumptive Identification of Aeromonas hydrophila and Enterobacteriaceae. Applied and Environmental Microbiology 38:1023-1026.

30. Kaysner, C. A., C. Abeyta, Jr., M. M. Wekell, A. DePaola, Jr., R. F. Stott and J. M. Leitch. 1987. Virulent strains of Vibrio vulnificus isolated from estuaries of the United States west coast. Applied and Environmental Microbiology 53(6):1349-1351.

31. Koburger, J. A. and M. L. Miller. 1985. Evaluation of a Fluorogenic MPN Procedure for Determining Escherichia coli in oysters. Journal of Food Protection 48(3):244-245.

32. Kovacs, N. 1956. Identification of Pseudomonas pyocyanea by the oxidase reaction. Nature 178:703.

33. Lennette, E. H. (Ed.) 1980. Manual of Clinical Microbiology. Third Edit. American Society for Microbiology, Washington, D.C.

34. Peixotto, S. S., G. Finne, M. O. Hanna, and C. Vanderzant. 1979. Presence, growth and survival of Yersinia enterocolitica in oysters, shrimp and crab. Journal of Food Protection, Vol 42(12):974-981.

35. Presnell, M. W. 1970. Cooperative Bacteriological Study of Commercial Practices of Oyster Harvesting and Processing in Alabama. Special Report GCWHL 71-1, Gulf Coast Water Hygiene Laboratory, Dauphin Island, Alabama.

36. Presnell, M. W. and C. B. Kelly. 1961. Bacteriological Studies of Commercial shellfish Operations on the Gulf Coast. U. S. Department of Health, Education and Welfare, Public Health Service, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

37. Reily, L. A., C. R. Hackney, T. E. Graham and D. M. Sbaih. 1985. Postharvest changes in the microbiological quality of shellstock Louisiana oysters. Proceedings of the Tenth Annual Tropical and Subtropical Fisheries Conference of the Americas 10:249-255.

38. Rodrick, G. E., M. A. Hood, and N. J. Blake. 1982. Human Vibrio gastroenteritis. Medical Clinics of North America 66:665-673.

39. Rutala, W. A., F. A. Sarubbi, Jr., C. S. Finch, J. N. MacCormack, and G. E. Steinkraus. 1982. Oyster-associated outbreak of diarrhoeal disease possibly caused by Plesiomonas shigelloides. Lancet, March 27:739.

40. Shotts, E. B., and R. Rimler. 1973. Medium for the isolation of Aeromonas hydrophila. Applied Microbiology 26:550-553.

41. Simonson, J. and R. J. Siebeling. 1986. Rapid serological identification of Vibrio vulnificus by Anti-H coagglutination. Applied and Environmental Microbiology 52(6):1299-1304.

42. Sobsey, M. D., C. R. Hackney, R. J. Carrick, B. Ray, and M. L. Speck. 1980. Occurrence of enteric bacteria and viruses in oysters. Journal of Food Protection 43(2):111-113.

43. Son, N. T. and G. H. Fleet. 1980. Behavior of pathogenic bacteria in the oyster, Crassostrea commercialis, during depuration, re-laying, and storage. Applied and Environmental Microbiology 40(6):994-1002.

44. Spite, G. T., D. F. Brown, and R. M. Twedt. 1978. Isolation of an enteropathogenic, Kanagawa-positive strain of Vibrio parahaemolyticus from seafood implicated in acute gastroenteritis. Applied and Environmental Microbiology 35:1226-1227.

45. Tamplin, M., G. E. Rodrick, N. J. Blake, and T. Cuba. 1982. Isolation and characterization of Vibrio vulnificus from two Florida estuaries. Applied and Environmental Microbiology 44(6):1466-1470.

46. Tepedino, A. A. 1982. Vibrio parahaemolyticus in Long Island oysters. Journal of Food Protection 45(2):150-151.

47. Thompson, C. A., C. Vanderzant and S. M. Ray. 1976. Relationship of Vibrio parahaemolyticus in oysters, water and sediment, and bacteriological and environmental indices. Journal of Food Science 41(1):117-122.

48. Thompson, C. A., C. Vanderzant and S. M. Ray. 1976. Effect of processing, distribution and storage on Vibrio parahaemolyticus and bacterial counts of oysters (Crassostrea virginica). Journal of Food Science 41(1):123-127.

49. Thomson, W. K. and C. L. Thacker. 1972. Incidence of Vibrio parahaemolyticus in shellfish from eight Canadian Atlantic sampling areas. Journal of the Fisheries Research Board of Canada 29:1633-1635.

50. Tison, D. L. and M. T. Kelly. 1986. Virulence of Vibrio vulnificus strains from Marine Environments. Applied and Environmental Microbiology 51(5):1004-1006.

51. Watkins, W. D., C. D. Thomas, and V. J. Cabelli. 1976. Membrane Filter Procedure for Enumeration of Vibrio parahaemolyticus. Applied and Environmental Microbiology 32(5):679-684.

52. Weissman, J. B., W. E. Dewitt, J. Thompson, C. N. Mushnick, B. L. Portnoy, J. C. Feeley, and E. J. Gangarosa. 1975. A case of cholera in Texas. American Journal of Epidemiology 100:487-498.

Dissemination of Information

Papers Presented at Meetings:

1. Cook, David W., Angela M. Dicharry and Daniel R. Zwerg
   Fate of Fecal Coliform Bacteria and Vibrio species in Shellstock Oysters.
   Thirtieth Annual Gulf and Atlantic States Shellfish Conference
   June 2, 1986
   Biloxi, Mississippi

2. Cook, David W.
   Accummulation and Fate of Microorganisms in Oysters
   Conference on Depuration Technology for the Louisiana Oyster Industry
   May 6, 1986
   Baton Rouge, Louisiana

3. Cook, David W., Angela Dicharry Ruple and Daniel R. Zwerg
   Role of Environmental Factors in the Multiplication of Bacteria in Post-Harvest Shellstock Oysters
   51st Annual Meeting of the Mississippi Academy of Sciences
   February 27, 1987
   Jackson, Mississippi

4. Cook, David W.
   Changes in Bacteria Levels in Commercially Shipped Oysters
   Louisiana Oyster Industry Convention
   August 8, 1987
   New Orleans, Louisiana

5. Ruple, Angela D. and David W. Cook
   Microflora Changes in Post-Harvest Shellstock Oysters
   First Joint Conference of the Tropical and Subtropical Fisheries Technology and Atlantic Fisheries Technology Societies
   November 9-11, 1987
   Orlando, Florida

Publications:

1. Cook, David W., Angela Dicharry Ruple and Daniel R. Zwerg. 1987. Role of Environmental Factors in the Multiplication of Bacteria in Post-Harvest Shellstock Oysters. Journal of the Mississippi Academy of Sciences, 1987 Abstracts, Volume XXXII Supplement, page 63.

2. Ruple, Angela D. and David W. Cook. 1987. Microflora Changes in Post-Harvest Shellstock Oysters. Proceedings of the 12th Annual Tropical and Subtropical Fisheries Conference of the Americas (in preparation).

3. Cook, David W. and Angela D. Ruple. Indicator Bacteria and Vibrio Multiplication in Post-Harvest Shellstock Oysters. Journal of Food Protection (in preparation).

Meetings Attended (other than listed under Papers Presented):

1. Gulf and South Atlantic States Shellfish Conference, Mobile, Alabama, June 1985.

2. Interstate Shellfish Sanitation Conference, Cherry Hill, New Jersey, August 1985.

3. Tropical and Subtropical Fisheries Technological Conference of the Americas, Tampa, Florida, January 13-16, 1986.

4. Shellfish Microbiology Reseach Workshop, Virginia Institute of Marine Science, Gloucester Point, Virginia, May 28-30, 1986.

ACKNOWLEDGEMENTS

The authors wish to express appreciation to the Louisiana oyster growers and harvesters who transported us to the harvest boats, graciously gave us permission to go aboard the boats to collect samples, and provided the oysters. Likewise, we express appreciation to the Mississippi oyster processors who transported oysters as part of the commercial handling studies.

We are deeply indebted to Dr. R. J Siebling and Ms. J. Simonson of Louisiana State University for providing the anti-H coagulation reagent used in the serological typing of the Vibrio cultures.

Financial support for this research was provided by the Mississippi-Alabama Sea Grant Consortium and the Gulf Coast Research Laboratory.

Technical assistance on this project was provided by Daniel R. Zwerg, Tina Casey and Cheryl Kerr. Secretarial support for preparing this report was provided by Mrs. Elizabeth Heal.